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Antibodies Against Enteroviruses in Intravenous Ig Preparations: Great Variation in Titres and Poor Correlation With the Incidence of Circulating Serotypes

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Antibody titres in immunoglobulin preparations for intravenous use were tested against 24 different enterovirus serotypes and varied between 1 : 100 and 1 : 10,000 within a single batch. Differences up to 8-fold were found for homologous titres between two different batches that were prepared 6 years apart. The lowest titre obtained was 1 : 40. The observed differences within and between the two batches could not be explained by different incidence of serotypes of enteroviruses circulating at the time blood was collected. Differences in titres of up to 18-fold were observed when several strains of the same serotype were tested suggesting that intratypic variation influences antibody titres.

It is concluded that immunoglobulin preparations contain antibodies against many enteroviruses, but that titres can be low and cannot be predicted from the incidence of any particular serotype circulating in the community. Because of intratypic variation, selection of a batch for specific treatment should be based on results obtained with the patient's own isolate, and not with a reference strain. ***J. Med. Virol. 53:273–276, 1997.***

KEY WORDS: neutralizing antibody; enteroviruses; intravenous immunoglobulins

INTRODUCTION

Enteroviral infections are common, especially in neonates and infants, and are usually asymptomatic or associated with nonspecific signs such as fever and flu-like symptoms. However, such infections are occasionally severe, or even fatal, causing myocarditis, hepatitis, meningitis, or meningoencephalitis. The host's constitution and the virulence of the circulating virus are main determinants of disease severity [Melnick, 1996], and antibodies are known to play a pivotal role in resolving infection. Thus, patients who suffer from hypo- or agammaglobulinaemia and who are at risk of chronic meningoencephalitis can be given effective prophylaxis with intravenous immunoglobulins (IVIG) [Galama, 1997; McKinney et al., 1987; Rotbart, 1995]. Once chronic meningoencephalitis becomes established, high doses of immunoglobulin must be given by the IV route and sometimes intrathecally. Ideally, immunoglobulins with a high titre of specific antibody against the strain involved should be given, but the virus cannot always be cultured from cerebrospinal fluid [Galama, 1997; Rotbart, 1995].

Neonates are also at risk of severe enteroviral disease [Cherry, 1995; Galama, 1997; Modlin, 1986]. They have subtle impairments of their host defenses and rely partly on maternally derived antibody for protection [Lewis and Wilson, 1996]. Indeed, there is some evidence that these antibodies can ameliorate infections [Modlin et al., 1981; Naginton et al., 1983] and mother-to-child transmission in the absence of specific antibody is considered to be a risk factor for severe disease [Galama, 1997; Modlin, 1986]. This provides the rationale for treating neonates with severe enteroviral infection with IVIG. However, the efficacy of this form of treatment has yet to be established [Abzug et al., 1995a; Johnston and Overall, 1989; Naginton et al., 1983], which is difficult because treatment has to be started promptly before any information becomes available on the specific antibody levels [Abzug et al., 1995a;1995b].

Immunoglobulin is prepared from a pool of several thousand blood donations, as it is generally accepted that this will provide broad-spectrum coverage against the common nonpolio enteroviruses, of which 65 different serotypes exist while minimising batch-to-batch variation. However, little is known about the specific antibody levels in these preparations. Therefore, we determined the amount of neutralizing antibody against 24 different serotypes of enterovirus in two

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batches of immunoglobulin that had been prepared 6 years apart and compared the antibody titres with the incidence of the different enteroviral serotypes that predominated in the Netherlands at the time the blood had been donated.

MATERIALS AND METHODS

Viruses

The nonpolio enteroviruses consist of 65 serotypes: 23 coxsackie A viruses (CAV), 6 coxsackie B viruses (CBV), 32 echoviruses (EV), and the enteroviruses (Ent), numbered 68–71 [Melnick, 1996]. The 20 serotypes that occurred in the Netherlands during 1979–1994 with an incidence of >1% were selected, based on 14,471 isolates, of which 10465 (72%) were serotyped. In addition, 4 serotypes were included that were found with an incidence of <1%, but had been reported as causing severe disease [Cherry, 1995; McKinney et al., 1987; Verboon-Maciolek et al., 1997]. Together, these 24 serotypes accounted for 90% of the isolates serotyped in the Netherlands during this period. Prototype strains of enteroviruses and some clinical isolates were obtained from the National Institute of Public Health and Environmental Protection (RIVM) in Bilthoven, the Netherlands and from our laboratory. Virus stocks were prepared by growing virus at low multiplicity of infection in buffalo green monkey (BGM) cells. Once the maximum cytopathic effect (CPE) was obtained, the entire culture was treated with chloroform [Kapsenberg, 1988], centrifuged at low speed and the supernates stored in small amounts at −80°C.

Virus Titration

Virus stocks were titrated using a microtitration method, involving 4-fold dilutions. Each dilution was tested in 8 wells of a 96-well plate containing BGM cells [van Kuppenveld et al., 1995]. The tissue culture infective dose ($TCID_{50}$) that resulted in a CPE in 50% of the cultures was calculated according to the method of Reed and Muench [1938].

Immunoglobulin Preparations

Immunoglobulin preparations were kindly provided by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB; Amsterdam, the Netherlands). Lot CLB-890628-675 had been collected during 1986–1988, and lot CLB-950518-H61 during 1992–1994. Each batch contained the pooled Ig fractions derived from 3,000–5,000 donor units. Ig batches were considered to be representative for the whole country, since blood had been collected at National Blood Centres and by additional campaigns conducted in those regions lacking a blood donation centre. The preparations were freeze-dried and stored at 4°C. Immediately before use, they were reconstituted using distilled water in accordance with the manufacturer's instructions to a concentration of 6 g/dl.

Neutralization Assay

A microneutralization assay was carried out in 96-well plates seeded with 10^4 BGM cells per well in 0.2 ml of minimal essential medium (MEM), containing sodium bicarbonate, antibiotics and 2% fetal bovine serum. The plates were incubated in a CO_2 incubator at 37°C. Virus stocks were diluted to a concentration of 2,000 $TCID_{50}$/ml. Reconstituted IG was diluted twofold from 1 : 4 to 1 : 2,048, or higher if needed in MEM containing HEPES buffer and antibiotics; 0.5 ml of Ig dilution was mixed with 0.5 ml of diluted virus suspension containing 1,000 $TCID_{50}$. These mixtures were then incubated for 1 hr at 37°C and overnight at 4°C. Next day, 0.1 ml medium was removed from each well and replaced by 0.1 ml of the virus-Ig mixtures. Each dilution was tested in 8 wells. The final virus dose was checked by backtitration of 18 control samples without antibody and was estimated to be a mean 46 ± 10 $TCID_{50}$/well (±2 SD). Neutralization reactions were read after 3 and 5 days and 50% endpoints were calculated according to the method of Reed and Muench [1938]. The precision of the neutralization assay was calculated for experiments using 6 different serotypes that were repeated on 7 different days; the mean difference in titre was found to be 1.69 ± 1.1 (±2 SD).

Epidemiology

The enteroviruses that had circulated in the community during 1979–1994 were held in the registry of the Netherlands Working Group on Clinical Virology, which involves nearly all virus diagnostic laboratories in the Netherlands, reporting results on a monthly basis. The number of diagnostic laboratories increased from 8 in 1979 to 15 in 1991, and each has a regional function so that the whole country is covered. Most of the laboratories make use of the same virus isolation and typing procedures [Kapsenberg, 1988], although the sensitivity of their techniques may have differed. Furthermore, asymptomatic and mild infections would have remained undetected since these cases are seldom referred for viral diagnosis. Therefore, the data reported provides only a rough estimate of the actual incidence of individual viral infections in the community.

RESULTS

Titres ranging from 1 : 40 to 1 : 10,720 were found against the various serotypes (Table I) and differences of up to 8-fold were noted between the two batches. There was no obvious correspondence between the levels of any particular antibody and the incidence of infection with that serotype in the Netherlands during 1979–1994. Comparison for two shorter periods of 5 years, starting shortly before, and ending after blood had been donated again failed to reveal any relationship between the titres and the incidence of the various viral infections current at the time. Several strains of serotypes CBV2, CBV3, E9, and E20 were tested to investigate a possible effect of intratypic varia-

TABLE I. Virus Strains, Incidence, and Titres of Neutralizing Antibody in IVIG

Serotype	Strain	%[a]	CLB-1989[c]	CLB-1995[c]
CAV 9	Bozek	3.0	1,450	2,690
CBV 1	Tucson	2.0	890	1,620
CBV 2	Ohio-1	2.8	3,550	2,880
	Pretorius		1,510	300
CBV 3	TvDee	4.3	340	2,690
	Nancy		1,230	150
CBV 4	Tilo	3.0	1,590	10,720
CBV 5	Dekking	3.5	1,740	260
EV 1	Farouk	1.1	210	480
EV 2[b]	Cornelis	0.3	150	450
EV 3	Morrisey	2.0	280	690
EV 5	Noyce	1.9	650	720
EV 6	D'Amori	4.5	630	590
EV 7	Wallace	3.2	1,450	1,620
EV 9	Hill	2.4	1,450	1,020
	Meyer		1,290	3,020
EV 11	Gregory	6.7	720	400
EV 14	Tow	2.4	200	230
EV 18	Metcalf	1.6	110	170
EV 19[b]	Burke	0.2	790	5,750
EV 20[b]	JV-1	0.9	340	350
	RIVM '83		40	100
	AZN '95		130	140
EV 22	Harris	5.2	1,120	6,610
EV 24[b]	DeCamp	0.3	50	100
EV 25	JV-4	2.5	720	1,910
EV 29	JV-10	1.5	100	100
EV 30	Bastianni	6.0	310	600
Ent 71	BRCR	1.3	500	1,660

[a]Percentages, based on 14,471 isolates obtained during 1979–1994 in the Netherlands.
[b]Low incidence, but reported to cause severe disease [Cherry, 1995; Verboon et al., 1997].
[c]Reciprocal values of titres, according to Reed and Muench.

TABLE II. Incidences for Entroviruses During the Time Periods of Blood Donation for IVIG

Period	1979–1994	1984–1988	1990–1994
Serotype			
CAV 9	3.0[a]	2.3	4.2
CBV 1	2.0	1.4	2.7
CBV 2[b]	2.8[b]	3.1[b]	2.7[b]
CBV 3[b]	4.3[b]	4.3[b]	6.3[b]
CBV 4[b]	3.0[b]	2.3[b]	3.4[b]
CBV 5	3.5[b]	4.3[b]	4.3[b]
EV 1	1.1	1.0	0.4
EV 2	0.3	0.4	0.2
EV 3	2.0	1.2	1.4
EV 5	1.9	0.2	0.9
EV 6	4.5	6.1	1.6
EV 7	3.2	3.6	3.2
EV 9	2.4	1.9	2.0
EV 11	6.7	6.3	6.1
EV 14	2.4	2.1	0.9
EV 18	1.6	1.5	2.4
EV 19[b]	0.2[b]	0.4[b]	0.1[b]
EV 20	0.9	0.6	1.2
EV 22[b]	5.2[b]	5.0[b]	7.4[b]
EV 24	0.4	0.3	0.1
EV 25	2.5	1.1	2.6
EV 29	1.5	1.0	3.1
EV 30	6.0	9.8	1.2
Ent 71	1.3	1.9	1.1
Number of isolates:	14,471	4,905	4,530

[a]Percentages of total numbers of enteroviruses, isolated in the Netherlands during periods as indicated.
[b]Serotypes for which >4-fold difference in titres was observed between batch CLB-1989 and batch CLB-1995.

tion which was indeed observed and resulted in differences of up to 18-fold in titres, particularly for CBV3 (Table I).

DISCUSSION

IVIG is used frequently to protect patients with Ig deficiency against infections caused by common pathogens such as enteroviruses [van der Meer and Zeegers, 1994]. Ig has also been used for the treatment of chronic meningoencephalitis caused by an enterovirus, but this treatment has little success. Even when given intrathecally, IVIG is rarely helpful, and the disease is fatal in most patients. Therefore, the emphasis is on prophylaxis, although there are no data in the literature to indicate how this can be achieved optimally. The dosage of IVIG for maintenance treatment has been increased during the past decades. The present recommendation in the Netherlands is to maintain IgG concentrations in serum of >5 g/L [van der Meer and Zegers, 1994], but an efficacy study with a much higher dose is in progress.

Ig preparations for intravenous use contain the pooled Ig fractions from at least 1,000 blood donations. Some manufacturers prepare even larger pools, containing ⩾20,000 units. It is conceivable that a large pool size will provide a more constant quality, with antibody specificities that reflect the incidences of common infections in the general population. To check this, we compared antibody titres of IVIG with the incidences of the corresponding enteroviruses in the Netherlands. Antibodies were detected against all 24 serotypes tested, but the titres for different serotypes varied up to 200-fold. These 24 serotypes comprised those that frequently circulate but included some also that were less frequent but that have been reported to cause severe disease. Unexpectedly, there was no relationship between antibody titres and the incidence of serotypes. This is illustrated by low antibody titres found for EV11 and EV30, which were frequently isolated throughout the observation period, and by an inverse relationship between the incidence of EV19 infection at different times and antibody titres (Tables I, II). This may be because the incidence data were based on clinical isolates obtained from symptomatic infections that predominate in small children or because the serotypes that circulate among children differ from those among adults.

The differences between the two batches were only up to 8-fold, which is in the range reported by Dagan et al. [1983]. By contrast, Abzug et al. [1995a] found no variation in titres between 2 lots of IVIG derived from batches 10 times larger than those of the CLB. This suggests that a large pool size may reduce variability still further. On the other hand, these investigators provided no information on the interval between blood donations for the lots they tested. We also observed considerable variation in titres when several strains of

the same serotype were tested, as did Abzug et al. [1995a]. Thus, the large pool size does not appear to compensate for intratypic variations, which might also explain why we failed to find any relationship between epidemiology and antibody titres, since prototype strains were employed, and only a few strains that had recently been isolated from patients.

Virus neutralization is a complex event in which different mechanisms are involved, such as aggregation of virus particles through antibody cross-linking, blocking of virus–receptor interaction, inhibition of cell penetration, or inhibition of uncoating [Mosser et al., 1989]. Furthermore, it was recently shown that variation exists between individual virus isolates within the same serotype of coxsackie B virus in the capacity to bind to different cell surface receptors [Bergelson et al., 1997]. Studies such as ours cannot test for all possible mechanisms of intratypic variation and will consequently have limitations. Hence, it remains the ideal to test IVIG preparations for antibody using the patient's own isolate despite the obvious difficulties involved. Nevertheless, our study has shown that antibody was at least present against all 24 serotypes employed. Some titres were low, but it is not known whether this might reduce therapeutic efficacy, nor does it give any indication of the titres that might be attained in a recipient. This aspect of immunoglobulin treatment for enterovirus infections has received little attention so far and will be the subject of a future study in patients suffering from agammaglobulinemia.

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